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Assessment of Recombinant Baculovirus-expressed Lassa Virus Nucleoprotein as a Serodiagnostics Antigen

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Abstract

Lassa virus is an arenavirus causing a disseminated systemic viral infection. This virus causes Lassa fever which is a viral hemorrhagic fever endemic in West Africa and is responsible for the death of thousands of people each year. There is a possibility for the Lassa virus to be introduced into the US and used as a biological weapon. This is of utmost concern with increased international travel, a sizable burden from the disease, and its potential use for bioterrorism. It is necessary to develop an efficacious, robust method to accurately detect virus infections and mitigate against potential introduction and spread. The objective of this project was to produce recombinant Lassa virus nucleoprotein (N), the most abundant and immunogenic structural protein of the virus, using a recombinant baculovirus expression system and testing its use as an antigen to detect Lassa antibodies in infected hosts. Using the expression system, the gene encoding for the N protein was cloned in a baculovirus vector, which was used to create a recombinant bacmid. The bacmid was used to transfect Spodoptera frugiperda (Sf9) cells to produce the recombinant baculovirus and express the target protein. The recombinant proteins were purified by affinity chromatography using nickel columns, and specific reactivity was assessed by western blot using anti-Lassa virus hyperimmune mouse ascitic fluid, mouse anti-Lassa monoclonal antibody 6G8, and mouse anti-Lassa monoclonal antibody 5000. An estimated 63kDa recombinant N protein was overexpressed and authenticated via specific reactivity with the anti-Lassa virus hyperimmune mouse ascitic fluid but not with the monoclonal antibodies. Manifestation of specific immunoreactivity with the recombinant N antigen suggests the activity of N protein to be recognized by both specific Lassa antibodies and thus possibly serve as a serodiagnostic antigen for detection and surveillance of Lassa fever in endemic and non-endemic regions. Future studies would entail evaluating the use of the recombinant antigen in ELISA to investigate the immunoprotection of Lassa fever among febrile patients in endemic countries in West Africa.

Cloning and Construction of Recombinant Bacmid

- The Lassa fever N gene was amplified from recombinant pUC57-LassaN plasmid by high fidelity PCR
- The PCR product was purified (Figure 3) and cloned into pFastBac plasmid creating a donor plasmid, pFastBac
- The plasmids were purified using the QiAprep Spin Miniprep Kit
- The accuracy of the N sequence in the recombinant pFastBac was confirmed by restriction enzyme analysis using Xhol and by DNA sequencing
- The recombinant pFastBac was used to create recombinant bacmid (Figure 4)

Lassa N Protein Expression and Purification

- Recombinant bacmid was used to transfect Spodoptera frugiperda (Sf9) cells to rescue a recombinant baculovirus
- Recombinant baculovirus passage 2 (P2) was used to express recombinant Lassa N protein in Sf9 cells
- Recombinant proteins were purified by affinity Ni-NTA column chromatography
- The purified protein was dialyzed overnight against PBS and concentration was measured using Nanodrop

Western Blot Analysis

- The purified protein was run through a polyacrylamide gel in 1X MOPS running buffer
- The protein was transferred onto PVDF membrane according to standard protocol
- The membranes were probed with anti-His (C-terminal)-HRP monoclonal antibody, and
- With monoclonal mouse Anti-Lassa antibodies and Anti-Lassa virus hyperimmune mouse ascitic fluid (1:2,000; 1:4,000; 1:8,000; 1:16,000)
- The membranes were probed with goat anti-mouse HRP (1:5000)
- Detection was performed using ECL chemiluminescent detection reagent and visualized using a bioRad imager.

Results

- Expression of recombinant Lassa virus N protein of the expected molecular size was detected using anti-His (C-terminal) monoclonal antibody
- Authenticity of the recombinant N protein was confirmed via specific reactivity with the virus hyperimmune ascitic fluid (Figure 5)
- The hyperimmune ascitic fluid detected an estimated 62 kDa protein which corresponded with the expected molecular size
- The recombinant protein exhibited specific reactivity anti-Lassa antibodies in high dilutions (up to 16,000-fold) of the hyperimmune ascitic fluid (Figure 6)
- High antibody concentration or activity appeared to correlate with signal intensity (Figure 6; lane 1 versus lanes 2-4)

References


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